Combinatorial Chemistry in Drug Research

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New Strategies in Drug Research

Targets from genomics and proteomics

Transgenic animals for “proof of concept”

Combinatorial chemistry

High-throughput screening (HTS, uHTS)

Virtual screening: Bioavailability rules, drug-like character

From protein 3D-structures to ligands
Disadvantages of Traditional Medicinal Chemistry

Complex and time-consuming syntheses
Low diversity (insufficient for new lead discovery)
Synthetic output too small
Slow development of structure-activity profiles within a class of compounds
Slow optimization in evolutionary cycles
Insufficient patent coverage
High costs (about 5,000 – 10,000 US-$ per compound)

Application of the Ugi Multicomponent Reaction
A library of therapeutically used local anesthetics

© L. Weber, Morphochem, Munich, Germany
Thrombin Inhibitors From an Ugi-Type Reaction

A 15,360-member library from
12 amines,
80 aldehydes,
16 isonitriles

K. Illgen et al.,
Chem. Biol. 7,
433-441 (2000)
Drug Space: Small Islands in a Huge Ocean

Lead Discovery: search an island
Lead Validation: explore the island
Lead Hopping: find adjacent islands
Lead Optimization: search for highest peaks

Chemogenomics is the systematic exploration of all islands
A Natural Product-Like Library \( (n = 2.18 \text{ mio}) \)

Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings

Christopher A. Lipinski*, Franco Lombardo, Beryl W. Dominy, Paul J. Feeney
Central Research Division, Pfizer Inc., Groton, CT 06340, USA
Received 9 August 1994; accepted 14 August 1994

Abstract

Experimental and computational approaches to estimate solubility and permeability in discovery and development settings are described. In the discovery setting, the rule of 5 predicts that poor absorption or permeation is more likely when there are more than 5 hydrogen donors, 10 hydrogen acceptors, the molecular weight (MWT) is greater than 500 and the calculated log P (LogP) is greater than 5 (MlogP = 4.15). Computational methodology for the rule-based Lipinski Log P (MlogP) calculation is described. Turbidimetric solubility measurement is described and applied to known drugs. High throughput screening (HTS) leads to have higher MWT and Log P and lower turbidimetric solubility than leads in the pre-HTS era. In the development setting, solubility calculations focus on exact value prediction and are difficult because of polymorphism. Recent work on linear free energy relationships and Log P approaches are critically reviewed. Useful predictions are possible in closely related analog series when coupled with experimental thermodynamic solubility measurements.
Development of Combinatorial Chemistry

- From peptides to organic molecules
- From large to small libraries
- From mixtures to single compounds
- From combinatorial synthesis to automated parallel syntheses of molecules with drug-like character

→ From chemistry to biological activity: focused design of combinatorial libraries

Goals: Search for new lead structures and optimization of their target affinity (= activity), selectivity, ADME properties (absorption, distribution, metabolism, elimination), reduction of toxicity and elimination of undesirable side effects.

Combinatorial Chemistry in Drug Research

Drug research is an evolutionary process
Nature developed higher organisms from more primitive forms.
Over the decades, lead structure search and optimization followed the same principles.

Combinatorial chemistry speeds up drug discovery
Automated parallel syntheses reduce the time needed for each evolutionary cycle.

Drug-like character of libraries
Biological properties are more important than synthetic accessibility.

Similarity and diversity
Similarity can be better defined than diversity, the “lack of similarity”.

Size and diversity of libraries
Huge libraries are most often a waste of time and resources, because of the time spent for chemistry optimization and limited diversity.
Combinatorial Chemistry in Drug Research

Combinatorial chemistry and rational drug design
Structure-based and computer-assisted design and virtual screening (LUDI, FlexX et al.) of protein ligands supplement combinatorial chemistry.

Combinatorial design of drugs
The necessary tools are already available but scoring functions have to be improved.

Success criteria in drug research
Decisive for industrial success is not "me too" but "me better", "me faster", "me first" or "me only".

Scope and limitations
Combinatorial chemistry and automated parallel synthesis will not replace classical chemistry.
They are powerful tools in lead discovery and optimization.

Types and Features of Combinatorial Libraries

Random libraries
- druglike
- diverse scaffolds

Chemogenomics

Targeted libraries
- target-directed
- diverse substitution
(target families)

Focused libraries
- similar to lead
- complete
Synthesis of a Peptoid Library

\[
\begin{array}{c}
\text{X} \\
\text{H}_3\text{C} - \text{CH}_2 - \text{OH} \\
\text{cHex} - \text{CH}_2 - \text{COOH} \\
\text{O} - \text{OH} \\
\text{O} - \text{OH} \\
\text{O} - \text{OH} \\
\text{X} \quad \text{R}_1 \quad \text{R}_2 \quad \text{R}_3 \\
\text{X} \quad \text{A} \quad \text{D} \quad \text{O} \\
\text{X} \quad \text{A} \quad \text{O} \quad \text{D} \\
\text{X} \quad \text{O} \quad \text{D} \quad \text{A} \\
\text{X} \quad \text{O} \quad \text{A} \quad \text{D} \\
\text{X} \quad \text{D} \quad \text{A} \quad \text{O} \\
\text{X} \quad \text{D} \quad \text{O} \quad \text{A} \\
\end{array}
\]

$K_i$ (α₁-adrenergic receptor) = 5 nM

$K_i$ (μ-specific opiate receptor) = 6 nM

**A Dihydropyridine Library (n = 300)**

- Split: 10 lots (10 synthons)
- Combine: a pool of 10 enamines
- Split: 30 lots (3 synthons) + Ar-CHO (10 synthons)
- Screening + Deconvolution: IC₅₀ (calcium channel) = 14 nM

**Synthesis of an ACE Inhibitor Library**

1. \( \text{CH}_2\text{Cl} + \text{Gly Ala Leu Phe} \rightarrow \text{AA:} \text{NH}_2 \text{NH} + \text{Ar-CHO:} \text{R} \)

2. \( \text{R:} \text{H Me OMe OSiMe}_2\text{Bu} \)

3. \( \text{Y:} \text{CN CO}_2\text{Me CO}_2\text{tBu COMe} \)

4. \( \text{R}_1: \text{H Me OMe OSiMe}_2\text{Bu} \)

5. \( \text{K}_i (\text{ACE}) = 160 \text{ pm} \)


**Solid Phase Synthesis of Epothilone, I**

1. \( \text{OH} \rightarrow \text{NaH, nBu}_4\text{I} \)

2. \( \text{PPh}_3, \text{I}_2, \text{imidazole} \)

3. \( \text{PPh}_3, 90^\circ\text{C} > 90\% \)

4. \( \text{HF/Py} \rightarrow \text{COCl}_2/\text{DMSO/TEA} \)

5. \( \text{ZnCl}_2, \text{THF} > 70\% \)

6. \( \text{LDA, } \text{THF} > 90\% \)

Solid Phase Synthesis of Epothilone, II

1. TFA/CH₂Cl₂
2. epoxidation

OH, DCC/DMAP

Grubbs catalyst
CH₂Cl₂, 48h, 52%

Epothilone A and isomers (separation and purification by HPLC and prep. TLC)
Synthesis of epothilone libraries

Dysidiolide Analog Libraries as cdc 25 Inhibitors

Dysidiolide
IC₅₀ cdc 25A = 9.4 µM

6-epi-Dysidiolide
IC₅₀ cdc 25A = 13 µM
IC₅₀ cdc 25B = 18 µM
IC₅₀ cdc 25C = 5.1 µM

6-epi-Dysidiolide analog libraries

D. Brohm et al., J. Am. Chem. Soc. 124, 13171-13178 (2002);
**Dysidiolide Analog Libraries as cdc 25C Inhibitors**


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<th>R</th>
<th>Structure</th>
<th>IC$_{50}$</th>
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**Serine Protease Inhibitors**

**Convergent Solid Phase Synthesis, Part I**

1) FMOC-D-Cha-OH, DIEA, TBTU, DMF
2) piperidine/DMF

P2 fragment

1) NO$_2$SO$_2$Cl, DIEA, CH$_2$Cl$_2$
2) Br, Cs$_2$CO$_3$, NMP
3) HOAc, CH$_2$Cl$_2$, TFE (1:3:1)

P2-P3 fragment

P2-P4 fragment
Serine Protease Inhibitors
Convergent Solid Phase Synthesis, Part II

1) P2-P4-fragment, DIC
2) PhSH, K$_2$CO$_3$
3) TFA, H$_2$O (95:5)

thrombin inhibitor, IC$_{50}$ = 3.6 nM


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Serine Protease Inhibitors
Convergent Solid Phase Synthesis, Results

2 FTE’s, 4 months reaction optimisation
Several nanomolar thrombin inhibitors

New Serine Protease Program (Organ Protection)

HTS of BASF library no hits
BASF thrombin inhibitors no hits

Homology modelling
Binding site hypothesis
SPS of 40 analogs (2 weeks) submicromolar hits
**Solid Phase Synthesis of Pyrrolidinodiketopiperazines**

1. TFA/DCM
2. TEA/DCM
3. TMOF, R2O

1. DBU or TEA, LiBr, THF
2. R2H

R2 = aryl, hetaryl
R3 = NR'R'', OR', alkyl, aryl

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**Hoffmann La Roche Neurokinin Receptor Ligands**

Spirohydantoins (upper row) are low-affinity ligands but selected spiropyrrolidino-pyrrolidines (lower row) show 5 to 8 nM affinities.

**Merck Lead Discovery Libraries, I**

- 5-HT₆: 0.7 nM
- 5-HT₇: 300 nM
- 5-HT₁₅: > 1 µM
- 5-HT₅α: > 1 µM

- NK₁: 0.8 nM
- NK₂: > 10 µM
- NK₃: > 10 µM

- NPY₅: 0.8 nM
- NPY₁: > 10 µM


**Merck Lead Discovery Libraries, II**

- MC₄R: 612 nM
- GnRH: 52 nM

- CCR₅: 1,190 nM
- CCR₁: > 10 µM
- CCR₂: > 10 µM
- CCR₃: 920 nM
- CXCR₃: > 10 µM
- CXCR₄: 1,520 nM

**Merck Somatostatin Mimics Library**  
(4 libraries, up to n = 350,000)


**Subtype Specificities of Somatostatin Mimetics**  
\(K_d\) values in nM

<table>
<thead>
<tr>
<th>Compound</th>
<th>sst1</th>
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<th>sst3</th>
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Random Chemistry: An Unbiased Approach to New Chemical Entities

\[ \text{NH}_2 \text{NH} \text{OH} \]

\[ \text{NH}_2 \text{NH} \text{O} \text{H} \]

\[ \text{CH}_3 \gamma \text{irradiation in different matrices} \]

\[ \text{NH}_2 \text{NH} \text{O} \text{CH}_3 \]

\[ \text{CH}_3 \text{HO} \text{N} \]

substrate of thymidine kinase

inhibitor of thymidine kinase


RECAP - a Retrosynthetic Combinatorial Analysis Procedure (applied to WDI)

Amide  Ester  Amine  Urea  Ether

Quaternary nitrogen  Aromatic N  Lactam N  Aromatic C  Sulphonamide
aliphatic C  aliphatic C  aromatic C

“Good Combinatorial Chemistry Practice“

Drug Design is an evolutionary procedure
Combinatorial chemistry speeds up drug discovery
Lead discovery libraries shall have a high degree of chemical diversity
Lead optimisation libraries shall have a high degree of similarity, to cover the chemical space around a lead structure
Several small libraries generate a higher diversity than one large library
Drug-like character is more important than synthetic accessibility

The Projectors of Speculative Learning of the Academy of Sciences of Lagado
Jonathan Swift
Gulliver’s Travels, 1726
Part III. A voyage to Laputa, Balnibarbi, Glubbdubdrib, Lugnagg and Japan.
Combinatorial chemistry is the synthesis of all possible combinations of chemical building blocks. When it first originated only a few years ago, the ability of this technology to generate millions of novel compounds seemed highly desirable. As scientists in the industry have grown more knowledgeable, however, it has become clear that the generation of such vast numbers of random compounds results in an overwhelming amount of unproductive work. Moreover, the range of synthetic organic reactions remained relatively small and identification of individual bioactive compounds was complicated. In addition, cell assays of mixtures were plagued with ambiguity, and mixtures frequently gave rise to meaningless positive responses.

Anthony W. Czarnik, Vice President Chemistry, IRORI, in Chemical & Engineering News, April 6, 1998, about large libraries:

"The motivating theme for combinatorial chemistry might be to be able to make a million variants of any structural template and screen them. Cheaper and faster are better. When we can accomplish this within a week, then perhaps the intellectual challenges will be gone. However, I can assure you that we are far, far from that vision today".

Mario Geysen, "Inventor" of Combinatorial Chemistry and Head of the Combinatorial Chemistry Group at Glaxo Wellcome (cited from G. Karet, Drug Discovery & Development, January 1999, pp. 32-38) about compound quantities in large libraries (1-2 ng):

"If you want to increase the numbers, the quantities have to be small; otherwise you are going to break the bank trying to make that library".